Unit 6
Molecular Biology

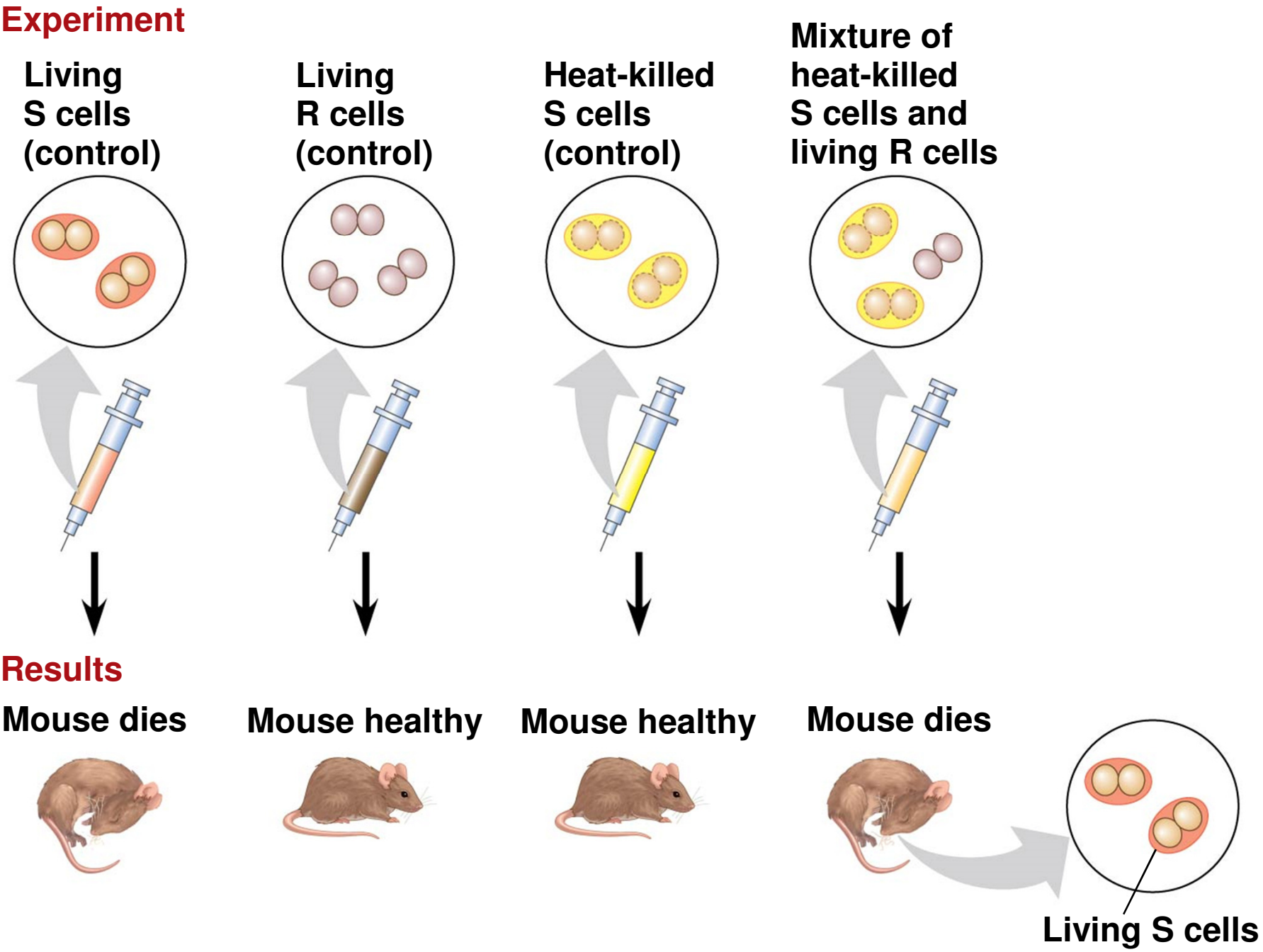
Chapter 13: The Molecular Basis of Inheritance

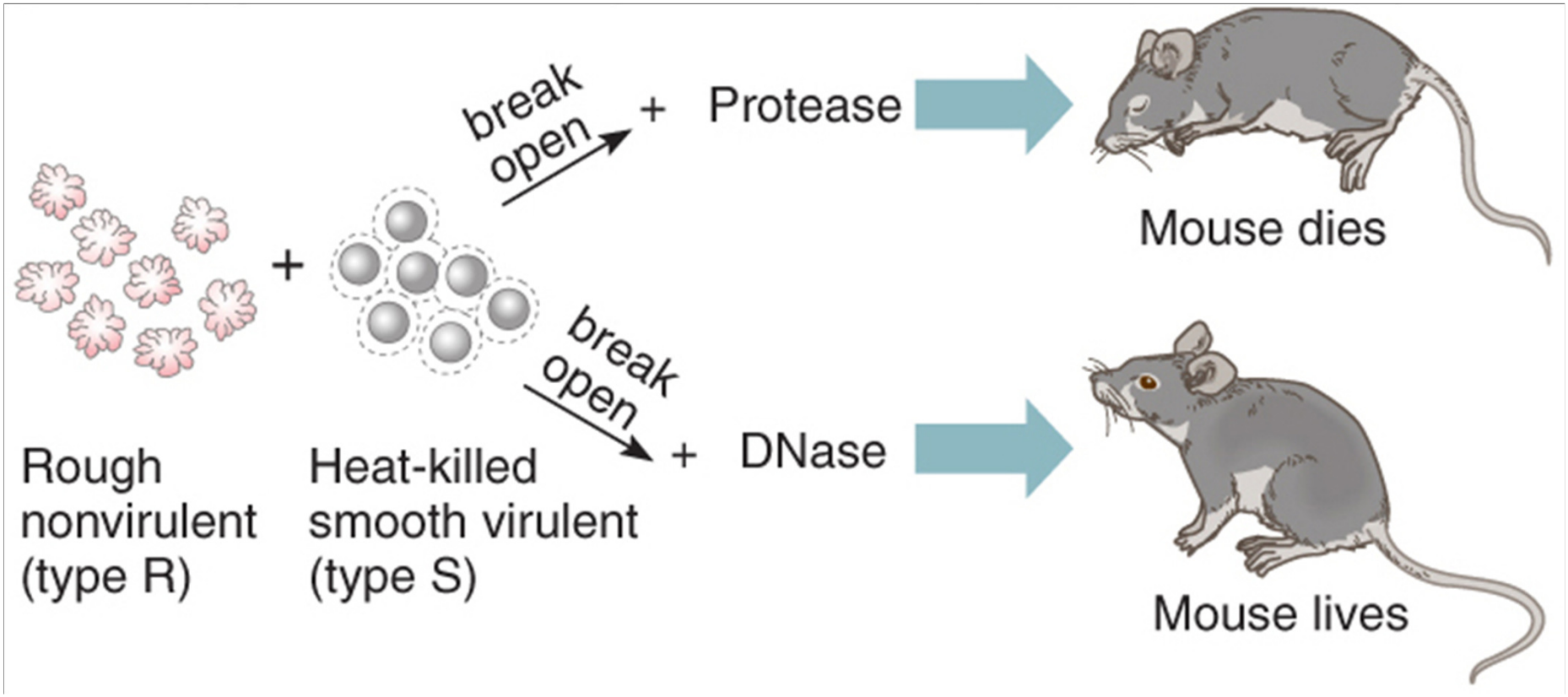
Evidence that DNA is the genetic material

Experiments:

- Frederick Griffith discovered **transformation**
  - Now defined as a change in genotype and phenotype due to assimilation of foreign DNA
  - Worked with two strains of a bacterium, one pathogenic and one harmless
  - When he mixed heat-killed remains of the pathogenic strain with living cells of the harmless strain, some living cells became pathogenic
- Oswald Avery and others identified the transforming substance as DNA

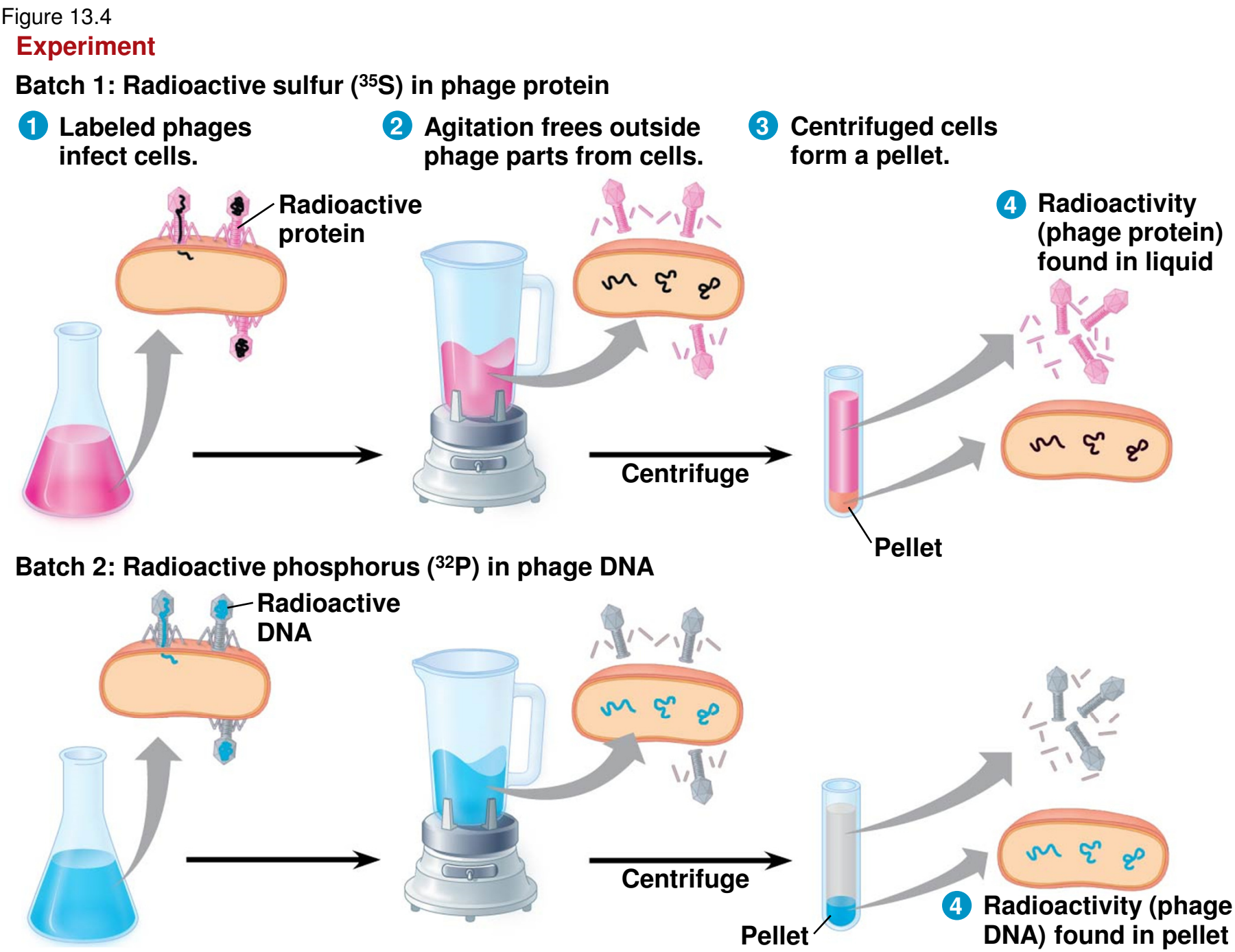
Figure 13.2


Experiment
Living S cells (control)
Living R cells (control)
Heat-killed S cells (control)
Mixture of heat-killed S cells and living R cells
Results
Mouse dies
Mouse healthy
Mouse healthy
Mouse dies
Living S cells


break open
+
Protease
Mouse dies
+
break open
+
DNase
Mouse lives
Rough nonvirulent (type R)
Heat-killed smooth virulent (type S)

- Hershey and Chase showed that DNA is the genetic material of a **bacteriophage**
  - Virus that infects bacteria
  - Used radioactive isotopes of
    - Sulfur to tag protein
    - Phosphorus to tag DNA
  - Showed that only the DNA, and not the protein, enters an *E. coli* cell during infection

Figure 13.4


Experiment
Batch 1: Radioactive sulfur (^{35}S) in phage protein
1 Labeled phages infect cells.
Radioactive protein
2 Agitation frees outside phage parts from cells.
3 Centrifuged cells form a pellet.
Centrifuge
Pellet
4 Radioactivity (phage protein) found in liquid
Batch 2: Radioactive phosphorus (^{32}P) in phage DNA
Radioactive DNA
Centrifuge
Pellet
4 Radioactivity (phage DNA) found in pellet

- It was known that DNA is a polymer of nucleotides, each consisting of 3 components:
  - A nitrogenous base
  - A sugar
  - A phosphate group
- Chargaff found that
  - The base composition of DNA varies between species
  - In any species the number of A and T bases is equal and the number of G and C bases is equal
  - EX: If 40% of a sample of DNA is adenine, how much is cystosine?

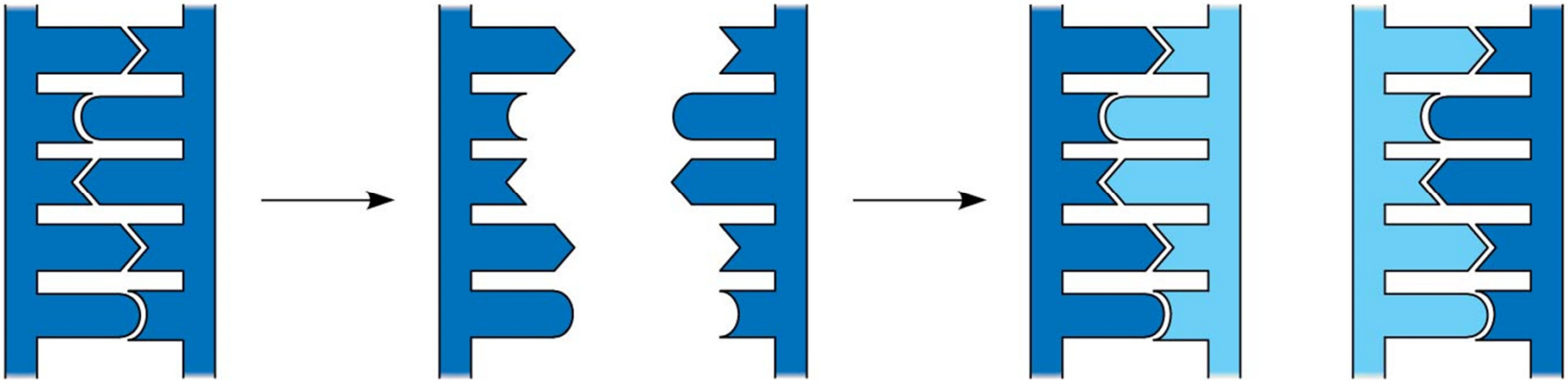
- Maurice Wilkins and Rosalind Franklin produced a picture of DNA using X-ray crystallography
- Those images led James Watson and Francis Crick were first to determine the structure of DNA is a **double helix**
  - Two outer sugar-phosphate backbones
    - **Antiparallel**
      - Their subunits run in opposite directions
  - Nitrogenous bases paired in the molecule's interior

- Purines have 2 organic rings
  - Guanine
  - Adenine
- Pyrimidines have single ring
  - Cytosine
  - Thymine
- They determined
  - Adenine (A) paired only with thymine (T)
  - Guanine (G) paired only with cytosine (C)
    - Explains Chargaff's rules!

DNA Replication

- DNA is copied during **DNA replication**
  - Occurs during S phase of interphase
- Since the two strands of DNA are ***complementary***, each strand acts as a template for building a new strand in replication
- In DNA replication, the parent molecule unwinds, and two new daughter strands are built based on base-pairing rules

- Watson and Crick's prediction was for a **semiconservative model** of replication
  - When a double helix replicates, each daughter molecule will have one old strand (derived or "conserved" from the parent molecule) and one newly made strand

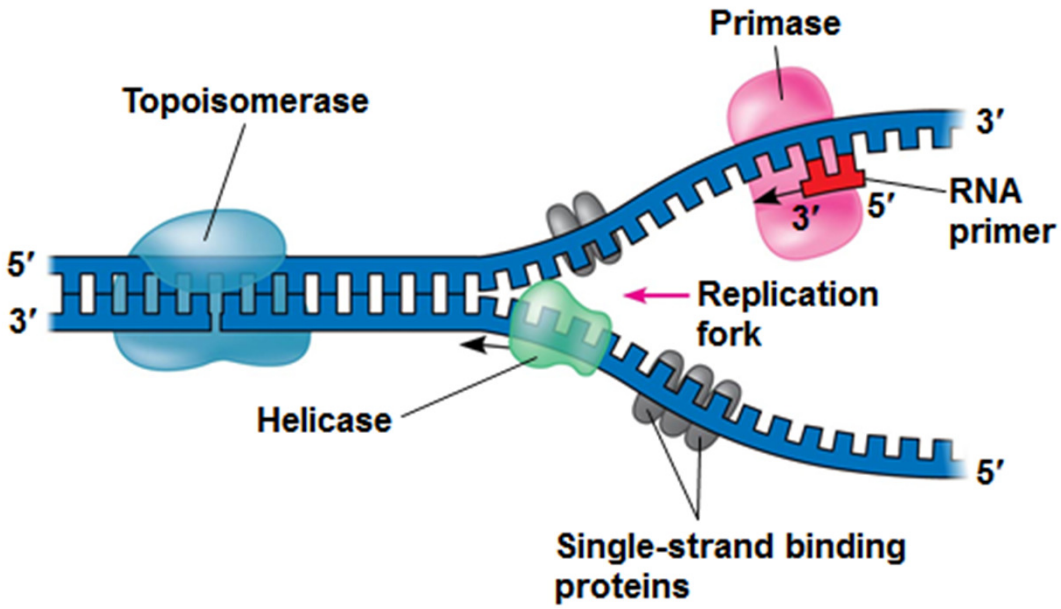


(a) Parental molecule

(b) Separation of parental strands into templates

(c) Formation of new strands complementary to template strands

- Replication begins at **origins of replication**
  - Here, the two DNA strands are separated, opening up a replication "bubble"
- At each end of a bubble is a **replication fork**
  - A Y-shaped region where the parental strands of DNA are being unwound



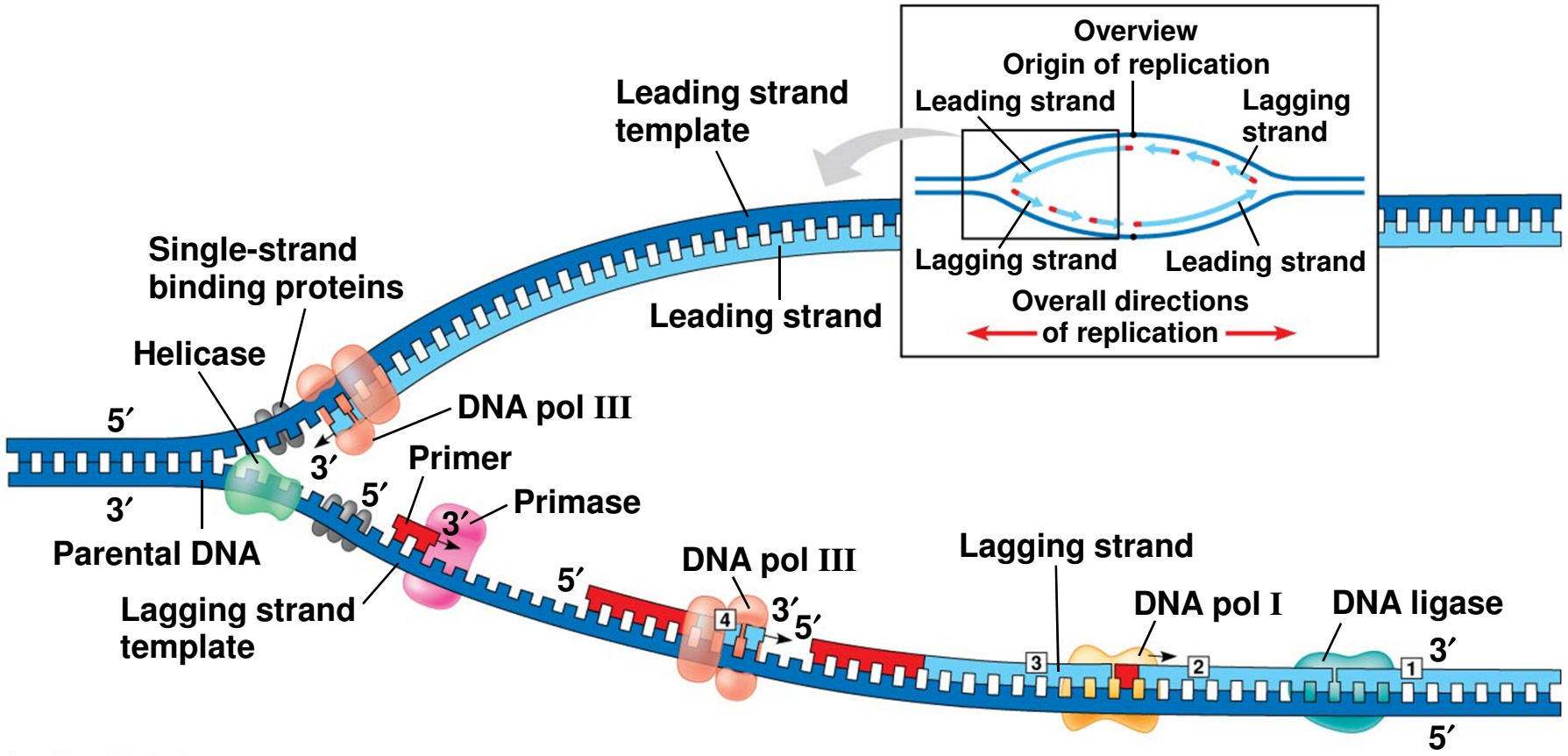
- Prokaryotes
  - Typically have one circular chromosome
  - Has single origin in replication, forming one replication bubble
  - Replication proceeds in both directions until entire molecule is copied
- Eukaryotes
  - Have hundreds to thousands of replication origins
  - Multiple replication bubbles form and eventually fuse
    - Speeds up the copying of DNA
  - Replication proceeds in both directions from each origin

Enzymes involved in DNA Replication

- **Helicases** are enzymes that untwist the double helix at the replication forks
- **Single-strand binding proteins** bind to and stabilize single-stranded DNA
- **Topoisomerase** relieves the strain caused by tight twisting ahead of the replication fork by breaking, swiveling, and rejoining DNA strands
- **Primase** adds a short RNA primer

- **DNA polymerase** catalyzes the elongation of the new DNA strand
  - Can only add nucleotides to the free 3′ end of a growing strand
  - Therefore, a new DNA strand can elongate only in the 5′ to 3′ direction!
    - **Leading strand** is synthesized continuously, moving toward the replication fork
    - **Lagging strand** is synthesized as a series of segments called **Okazaki fragments**
      - Because DNA is **antiparallel**!
- **DNA ligase** joins the fragments together

Figure 13.17


Overview
Origin of replication
Leading strand
Lagging strand
Lagging strand
Leading strand
Overall directions of replication
Leading strand template
Leading strand
Single-strand binding proteins
Helicase
DNA pol III
5′
3′
Primer
Primase
3′
5′
3′
Parental DNA
Lagging strand template
5′
DNA pol III
4
3′
5′
Lagging strand
DNA pol I
DNA ligase
3
2
1
3′
5′

Genetic Engineering

- **Genetic engineering** = the direct manipulation of genes for practical purposes
  - Applications include agriculture, criminal law, medical research
- **Polymerase chain reaction**, or **PCR**
  - Can produce many copies of a specific target segment of DNA
    - Fast and specific
  - Used to provide specific DNA fragment to be cloned

- **Recombinant DNA**
  - DNA from one source is inserted into a bacterial **plasmid**
    - Small circular DNA molecule that replicates separately from the bacterial chromosome
  - Note: Works because genetic code is universal

- Bacterial **restriction enzymes** cut DNA molecules at specific DNA sequences called **restriction sites**
  - Each restriction enzyme if very SPECIFIC
  - The same enzyme is used to cut the “foreign” DNA and plasmid DNA at the same restriction site
  - Cut in a staggered manner to produce **sticky ends**
    - Can bond with complementary sticky ends of other fragments
- DNA ligase seals the 2 pieces of DNA together

Figure 13.23-3

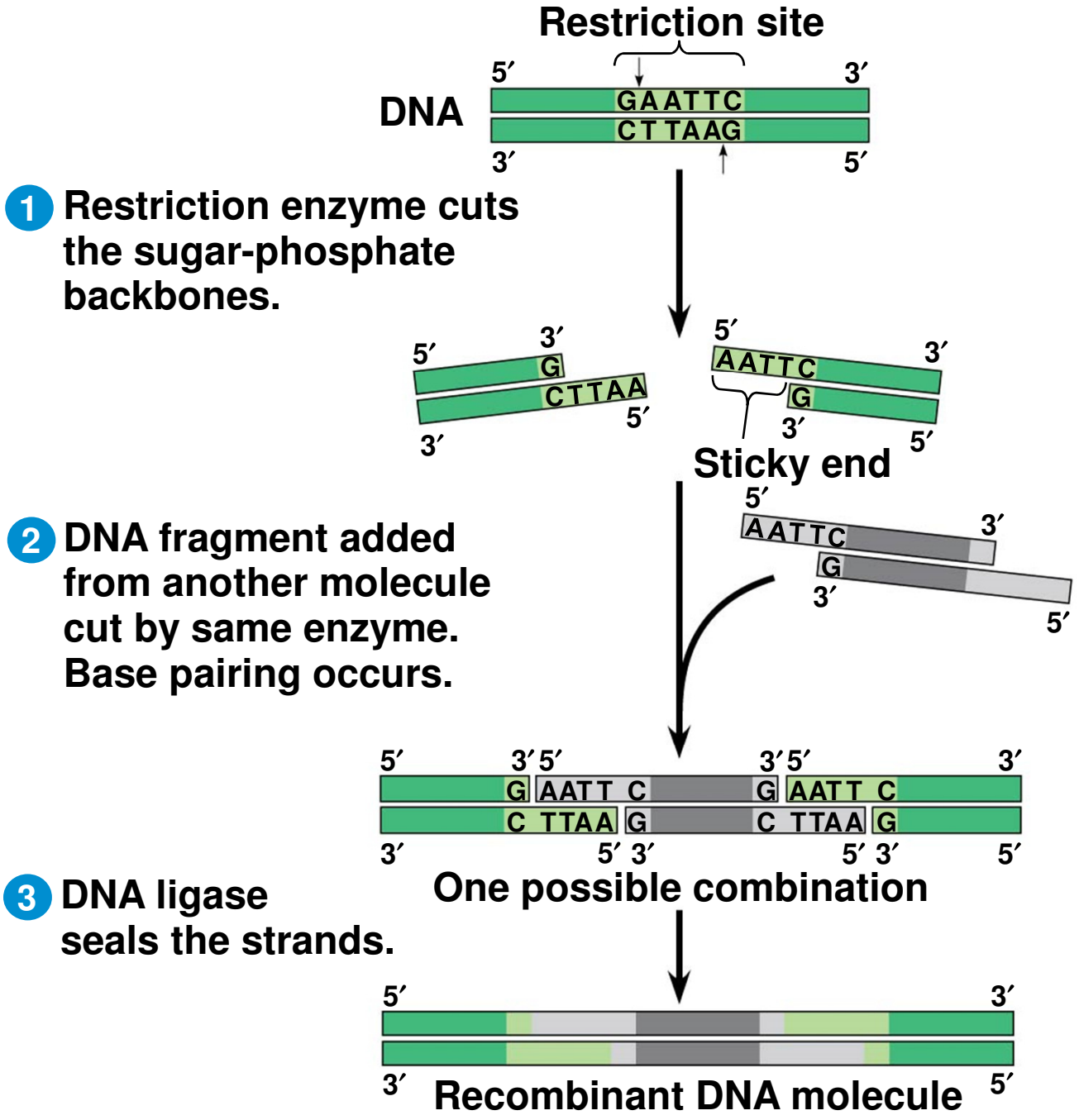


Figure 13.26

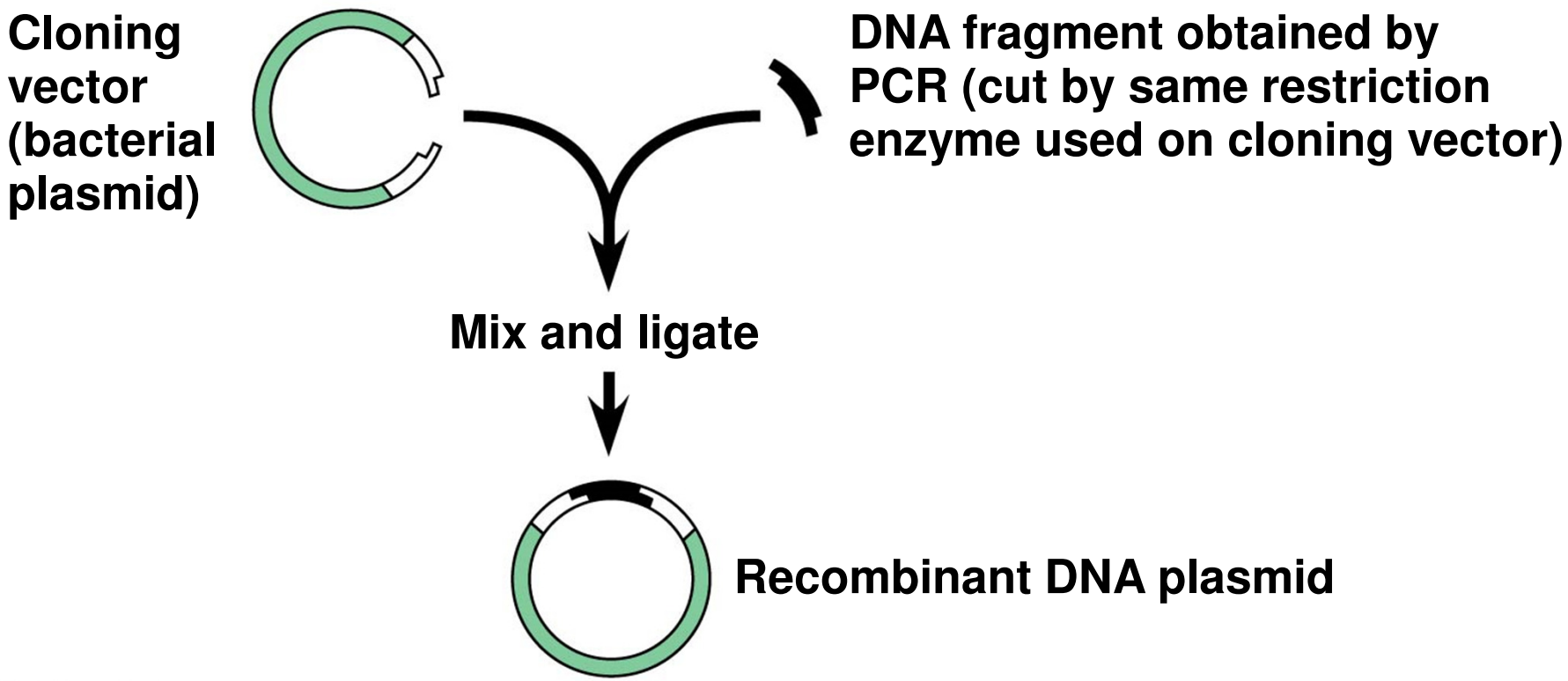
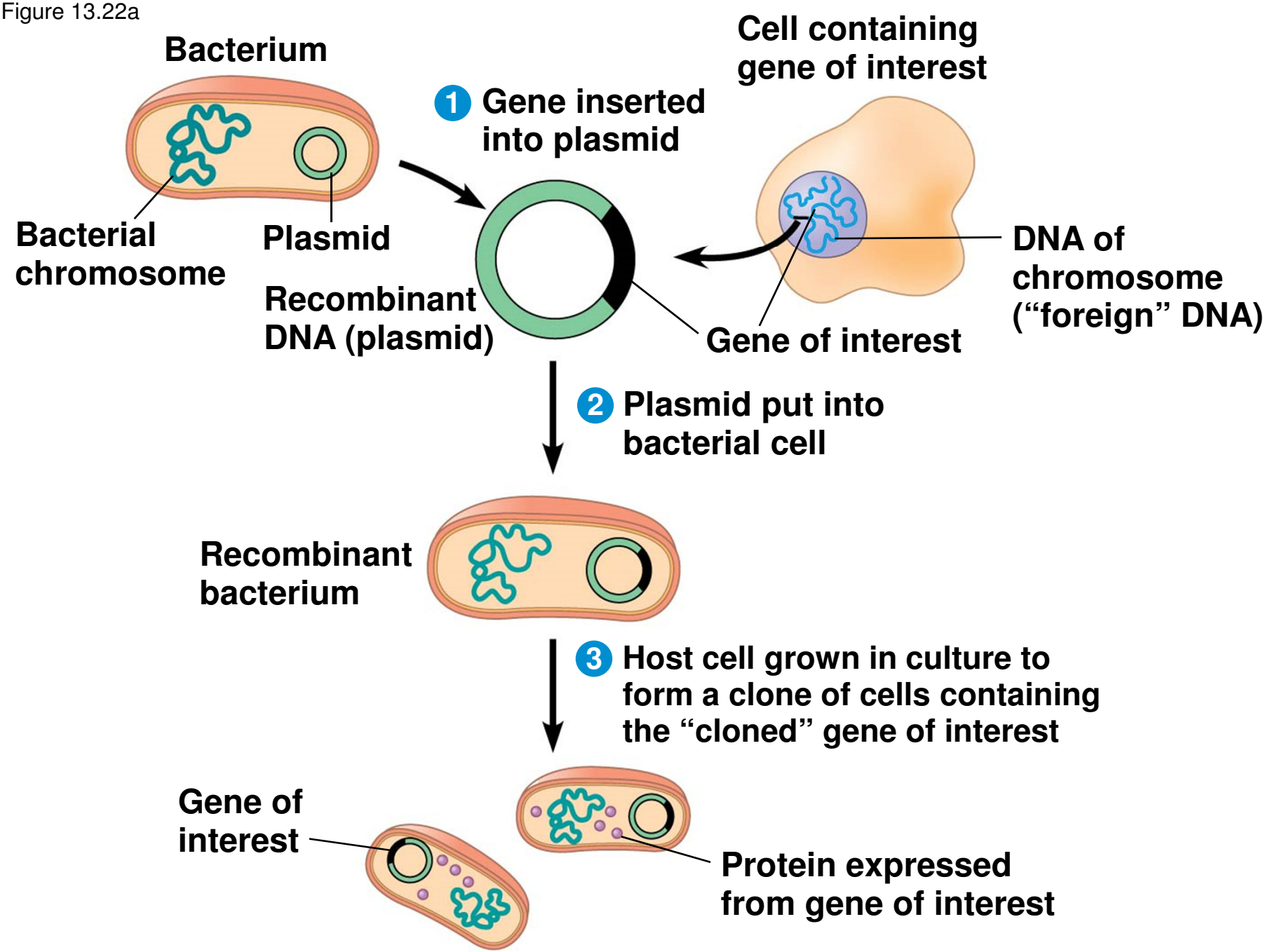
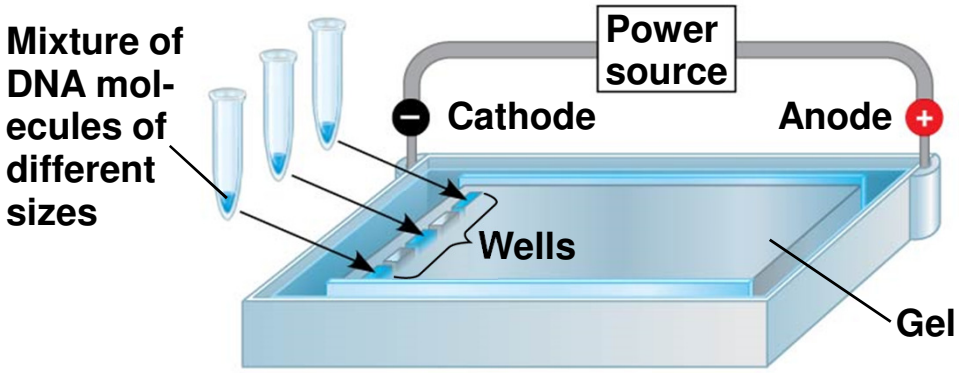

Cloning vector (bacterial plasmid)
DNA fragment obtained by PCR (cut by same restriction enzyme used on cloning vector)
Mix and ligate
Recombinant DNA plasmid

Figure 13.22a

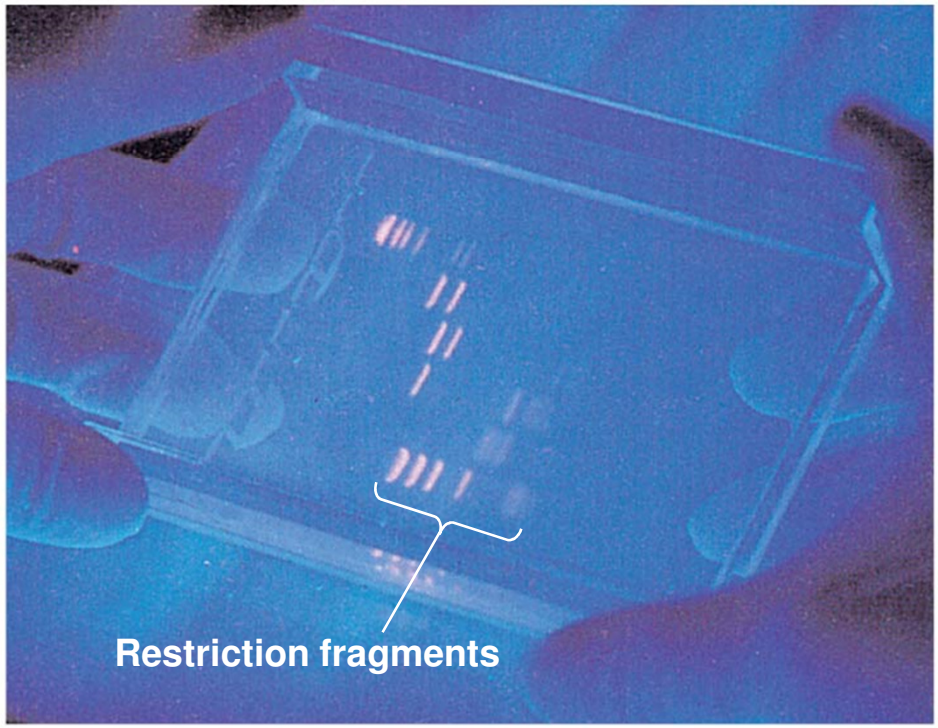


- **Gel electrophoresis**
  - Used to see the fragments produced by cutting DNA molecules with restriction enzymes, researchers use
  - This technique separates a mixture of nucleic acid fragments based on length
    - Current is applied to move fragments
      - Negatively charged DNA is loaded in wells at negative end because DNA will move to positive end
    - Smaller fragments move faster (and thus further) than large

Figure 13.24



(a) Negatively charged DNA molecules will move toward the positive electrode.



(b) Shorter molecules are impeded less than longer ones, so they move faster through the gel.

Unit 6
Molecular Biology

Chapter 14: Gene Expression:

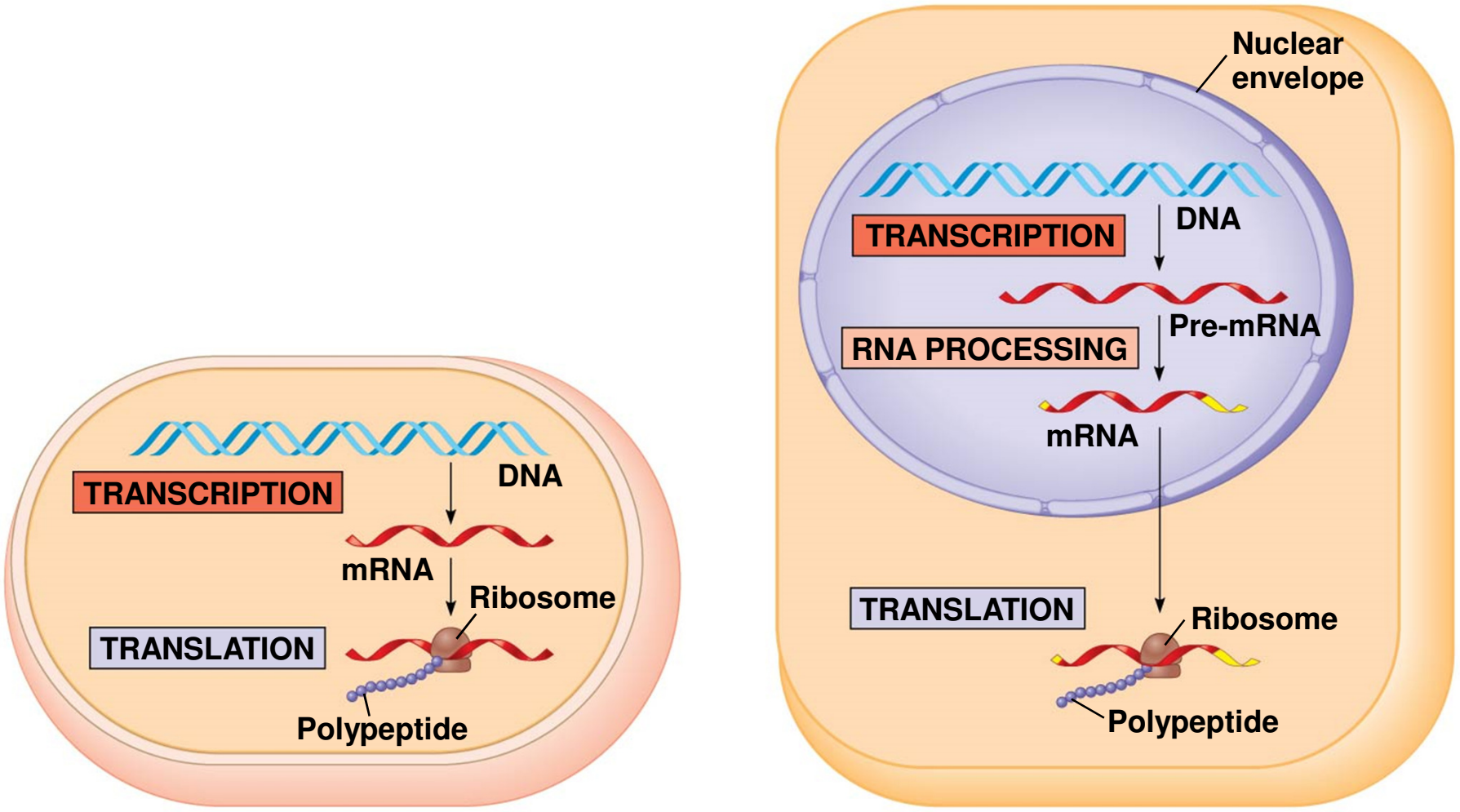
From Gene to Protein

Gene Expression

- Genes provide the instructions for making proteins
- Proteins are link between genotype and phenotype
- **Gene expression** is the process by which DNA directs protein synthesis
- Getting from DNA to protein requires two stages:
  - **Transcription** is the synthesis of **mRNA** using information in DNA
    - Occurs in nucleus of eukaryotes
  - **Translation** is the synthesis of a protein, using information in the mRNA
    - Occurs in cytoplasm at ribosomes

- RNA is the bridge between DNA and protein synthesis
- RNA is chemically similar to DNA, but RNA
  - Has a ribose sugar instead of deoxyribose
  - Has the base uracil (U) rather than thymine (T)
  - Is usually single-stranded

Figure 14.4

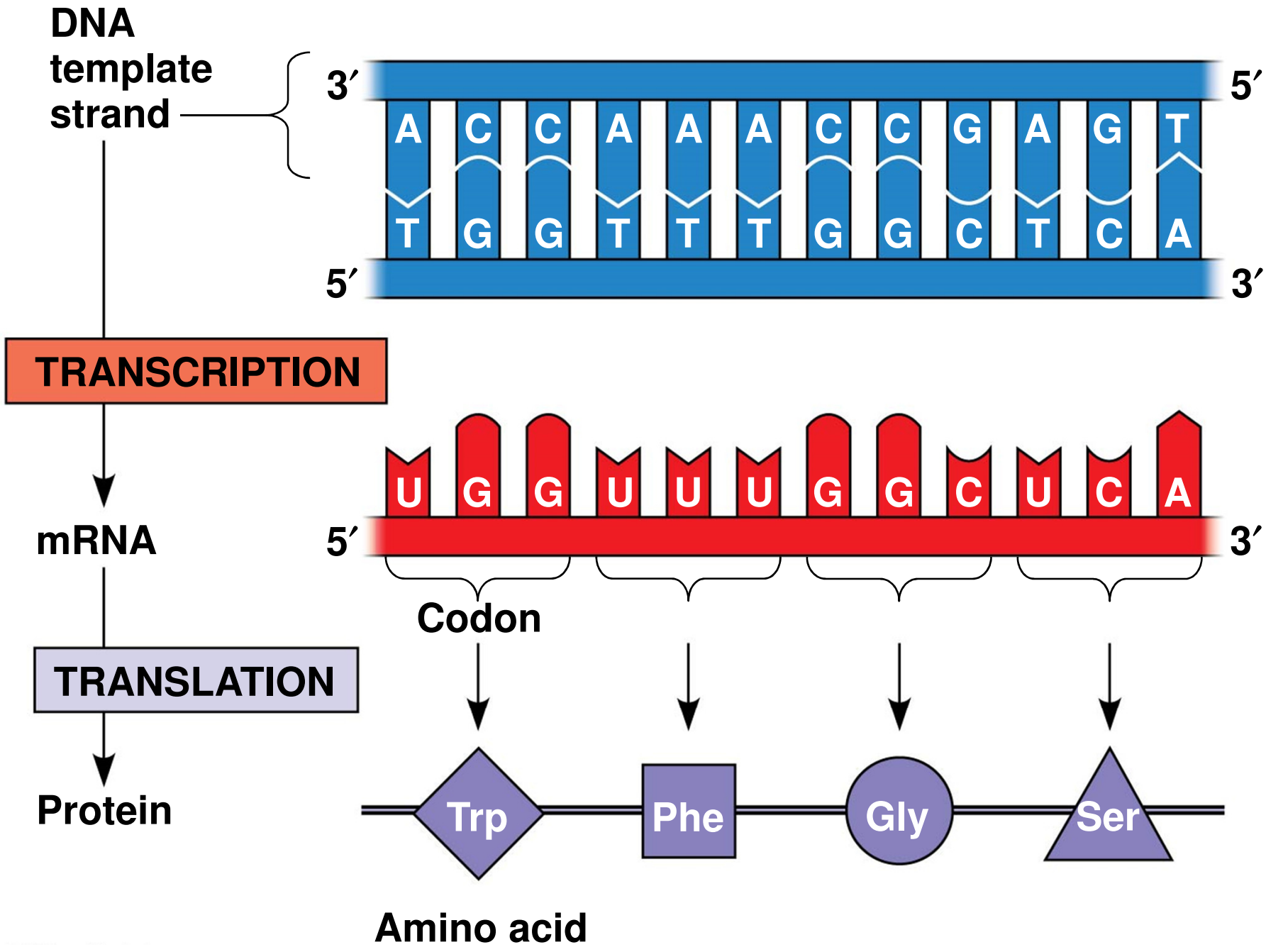


(a) Bacterial cell

(b) Eukaryotic cell

- The flow of information from gene to protein is based on a **triplet code**
  - A series of nonoverlapping, three-nucleotide words
- The words of a gene are *transcribed* into complementary nonoverlapping three-nucleotide words of mRNA
  - mRNA base triplets are called **codons**
- These words are then *translated* into a chain of amino acids, forming a polypeptide (protein)
- The genetic code is nearly universal
- Genes can be transcribed and translated after being transplanted from one species to another

Figure 14.5


DNA
template
strand
3′
5′
A C C A A A C C G A G T
T G G T T T G G C T C A
5′
3′
TRANSCRIPTION
mRNA
5′
3′
U G G U U U G G C U C A
Codon
TRANSLATION
Protein
Trp
Phe
Gly
Ser
Amino acid

Transcription and Translation Practice

- DNA template strand

  3’ TACGGTCGTTCGAATATC 5’

- mRNA codons

  5’ AUG CCA GCA AGC UUA UAG 3’

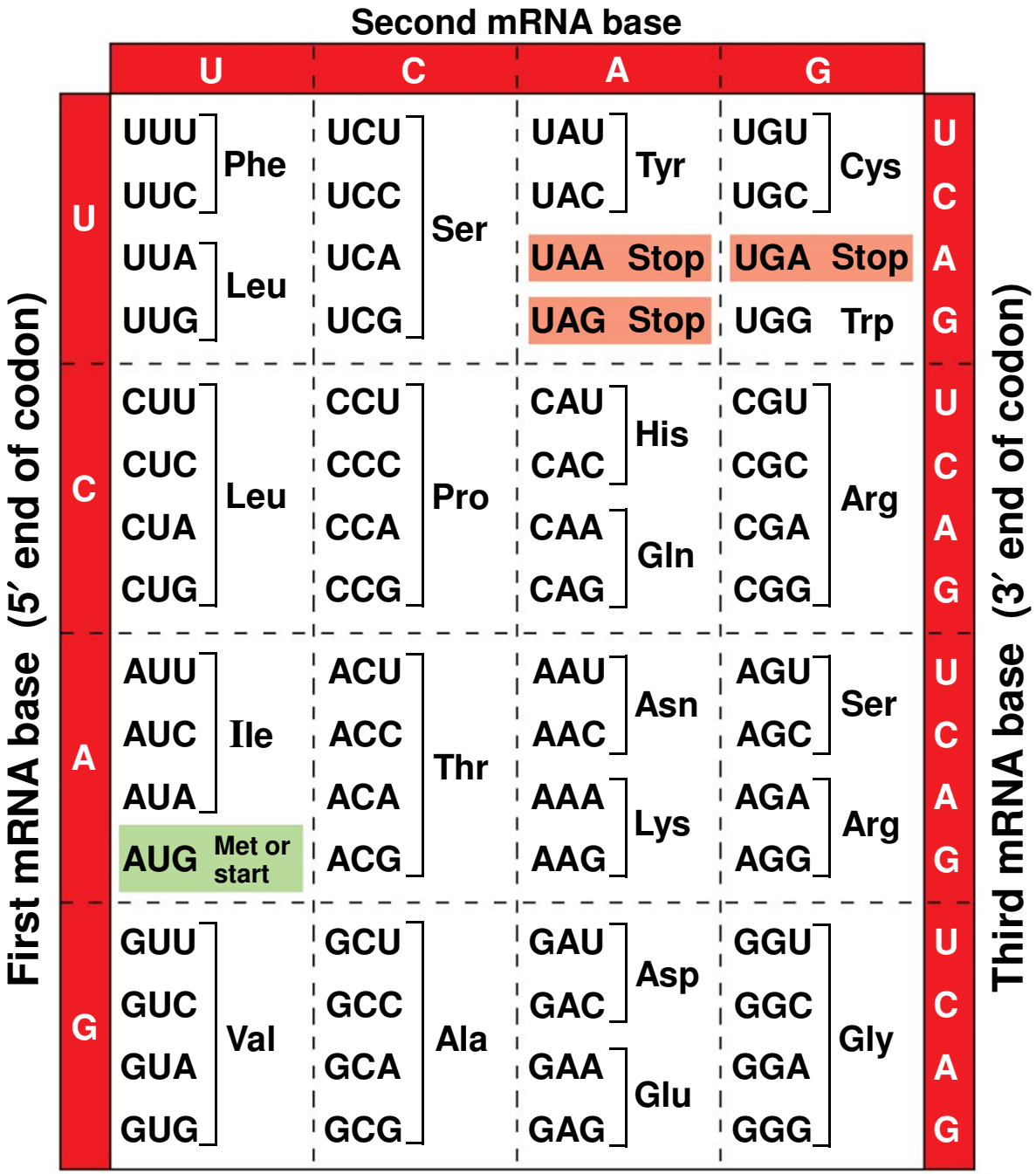
- tRNA anticodons

  UAC GGU CGU UCG AAU AUC

- Amino Acid (remember to use the codons!)

  Met Pro Ala Ser Leu (STOP)

Figure 14.6


Second mRNA base
U
C
A
G
First mRNA base (5′ end of codon)
Third mRNA base (3′ end of codon)
U
C
A
G
UUU Phe
UUC
UUA Leu
UUG
UCU Ser
UCC
UCA
UCG
UAU Tyr
UAC
UAA Stop
UAG Stop
UGU Cys
UGC
UGA Stop
UGG Trp
CUU Leu
CUC
CUA
CUG
CCU Pro
CCC
CCA
CCG
CAU His
CAC
CAA Gln
CAG
CGU Arg
CGC
CGA
CGG
AUU Ile
AUC
AUA
AUG Met or start
ACU Thr
ACC
ACA
ACG
AAU Asn
AAC
AAA Lys
AAG
AGU Ser
AGC
AGA Arg
AGG
GUU Val
GUC
GUA
GUG
GCU Ala
GCC
GCA
GCG
GAU Asp
GAC
GAA Glu
GAG
GGU Gly
GGC
GGA
GGG

Transcription = DNA → RNA

- Transcription is the first stage of gene expression
  1. Initiation
  2. Elongation
  3. Termination
- RNA synthesis is catalyzed by **RNA polymerase**
  - Assembles nucleotides in the 5′ to 3′ direction
- The DNA sequence where RNA polymerase attaches is called the **promoter**
- In bacteria, the sequence signaling the end of transcription is called the **terminator**

Initiation

- Promoters signal the transcriptional **start point**
- In eukaryotes, **transcription factors** mediate the binding of RNA polymerase and the initiation of transcription
- A promoter called a **TATA box** is crucial in forming the initiation complex in eukaryotes

Elongation

- mRNA is synthesized in the 5’ to 3’ direction
- As RNA polymerase moves along the DNA, it adds nucleotides to the 3’ end of the growing RNA molecule (*complementary* to DNA bases)
  - DNA has G; RNA pairs C
  - DNA has C; RNA pairs G
  - DNA has T; RNA pairs A
  - *DNA has A; RNA pairs U*

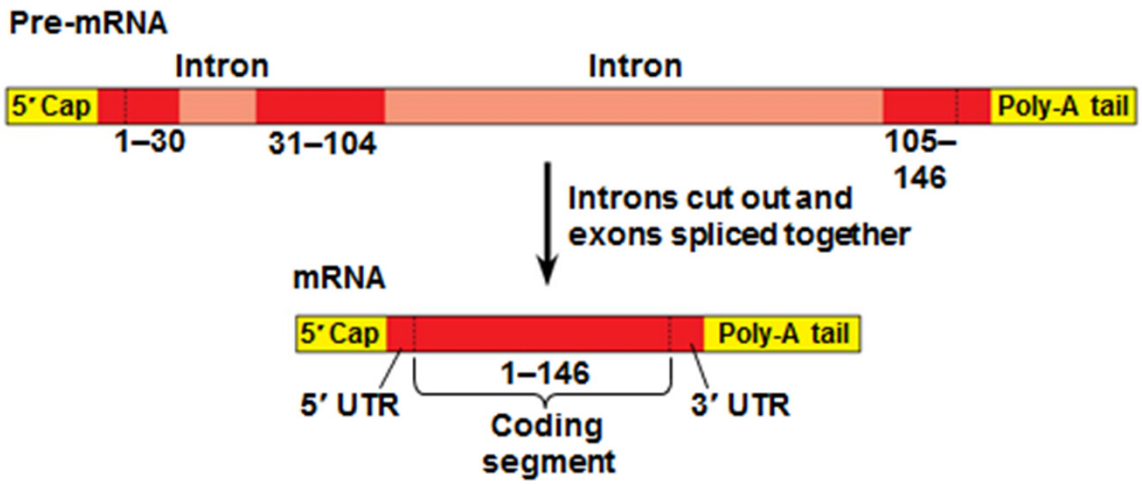
Termination

- The mechanisms of termination are different in bacteria and eukaryotes
  - In bacteria
    - The polymerase stops transcription at the end of the terminator
    - mRNA can be translated without further modification
  - In eukaryotes
    - RNA polymerase II transcribes the polyadenylation signal sequence
    - The RNA transcript is released 10–35 nucleotides past this polyadenylation sequence

RNA Processing

- In prokaryotes, translation directly follows transcription
  - Steps are not separated by a nucleus
- In eukaryotes, the mRNA is modified after being transcribed before it can be translated
  - **RNA processing**
- Each end of a pre-mRNA molecule is modified in a particular way
  - The 5′ end receives a modified **5′ cap**
  - The 3′ end gets a **poly-A tail**

- Most eukaryotic mRNAs have long noncoding stretches of nucleotides that lie between coding regions
- The noncoding regions are called intervening sequences, or **introns**
- The other regions are called **exons** and are usually translated into amino acid sequences
- **RNA splicing** removes introns and joins exons



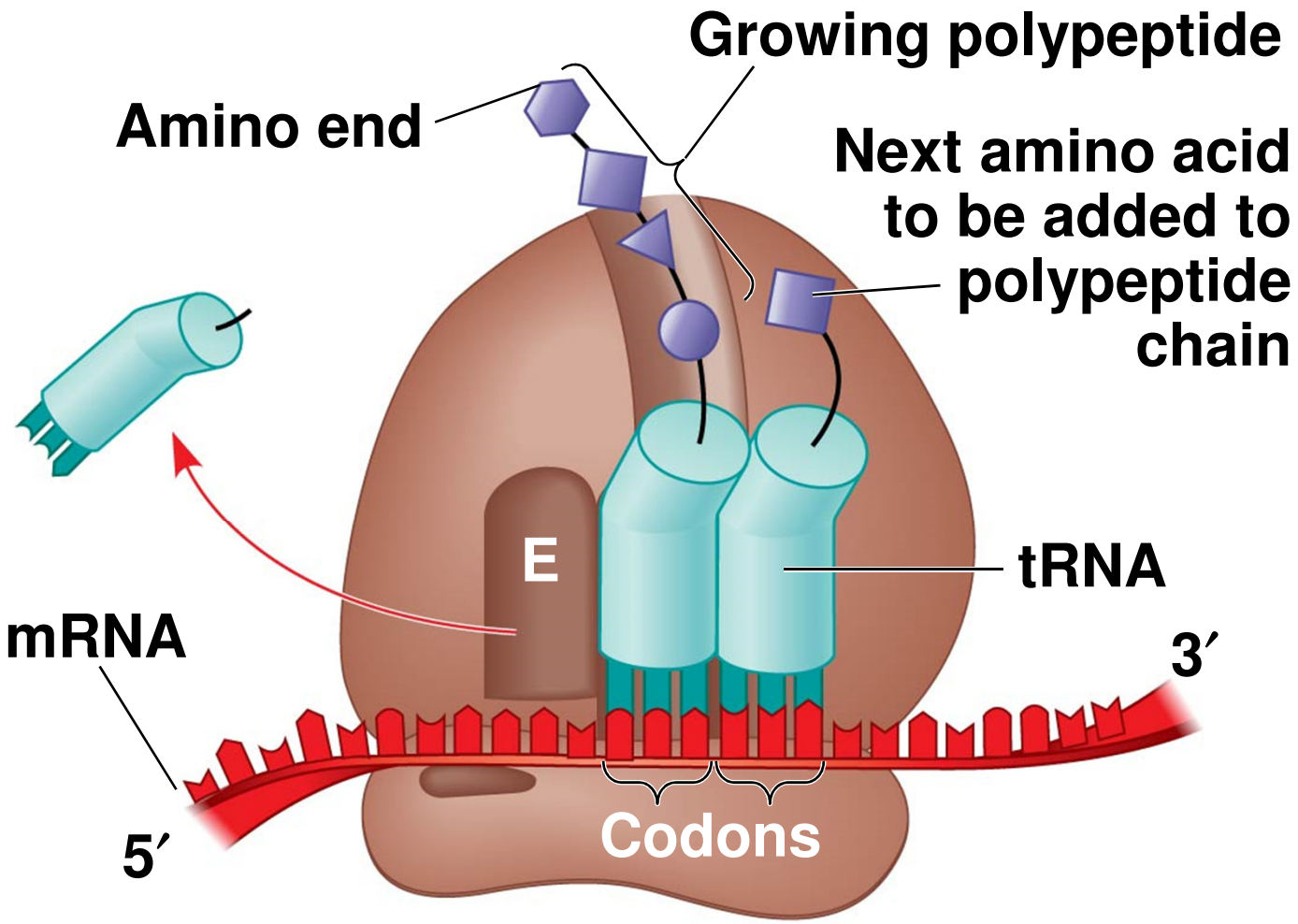
- Many genes can give rise to two or more different polypeptides
  - Depending on which segments are used as exons
  - This process is called **alternative RNA splicing**
    - Because of this, the organism's number of different protein products can be much greater than it's number of genes
- RNA splicing is carried out by **spliceosomes**
  - Complex of proteins and small RNAs that remove introns

Translation = RNA → Protein

- Genetic information flows from mRNA to protein through the process of translation
- A cell translates an mRNA message into protein with the help of **transfer RNA** (**tRNA**)
  - tRNAs transfer amino acids to the growing polypeptide in a ribosome
- One end of the tRNA contains the **anticodon**
  - Nucleotide triplet that pairs with an mRNA codon
- tRNA is a translator
  - Reads a nucleic acid word (mRNA codon)
  - Interprets it as a protein word (amino acid)

- Translation occurs at the ribosomes
  - The large and small ribosomal are made of proteins and **ribosomal RNAs (rRNAs)**
    - Made in nucleolus of eukaryotes
- A ribosome has three binding sites for tRNA
  - The **P site** holds the tRNA that carries the growing polypeptide chain
  - The **A site** accepts the tRNA that carries the next amino acid to be added to the chain
  - The **E site** is the exit site, where discharged tRNAs leave the ribosome
- tRNA moves from A to P to E sites

Figure 14.17c



(c) Schematic model with mRNA and tRNA

Mutations

- **Mutations** are changes in genetic material (DNA)
- **Mutagens** are physical or chemical agents that can cause mutations
  - Most cancer-causing chemicals (carcinogens) are mutagenic, and vice-versa
  - Radiation (X-rays and UV rays)
- **Point mutations** are chemical changes in just one or a few nucleotide pairs of a gene

Substitutions

- A **nucleotide-pair substitution** replaces one nucleotide and its partner with another pair of nucleotides
- **Silent mutations** have no effect on the amino acid produced by a codon because of redundancy in the genetic code
- **Missense mutations** still code for an amino acid, but not the correct amino acid
- **Nonsense mutations** change an amino acid codon into a stop codon
  - Nearly always lead to a nonfunctional protein

Figure 14.26a

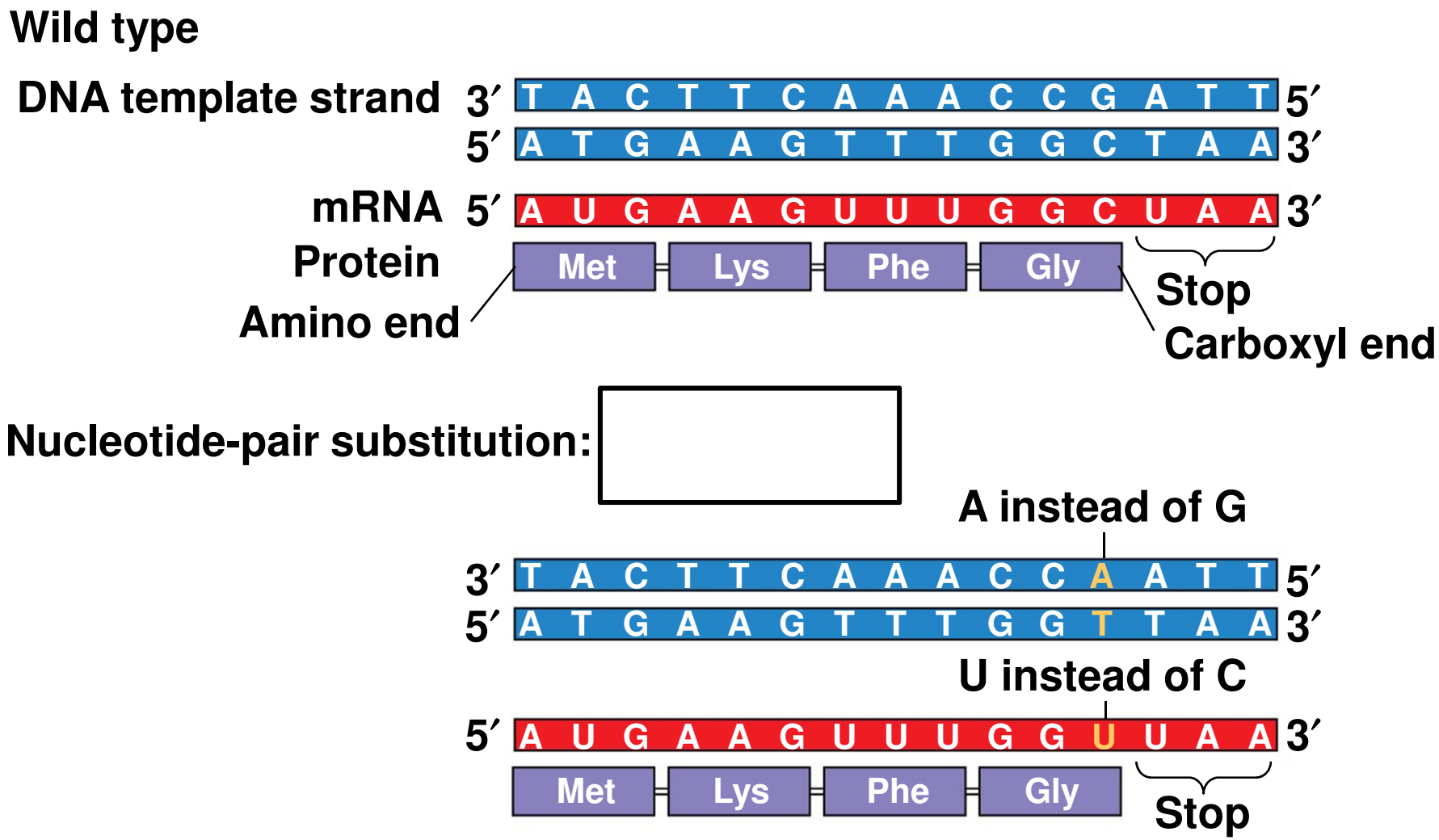


Figure 14.26b

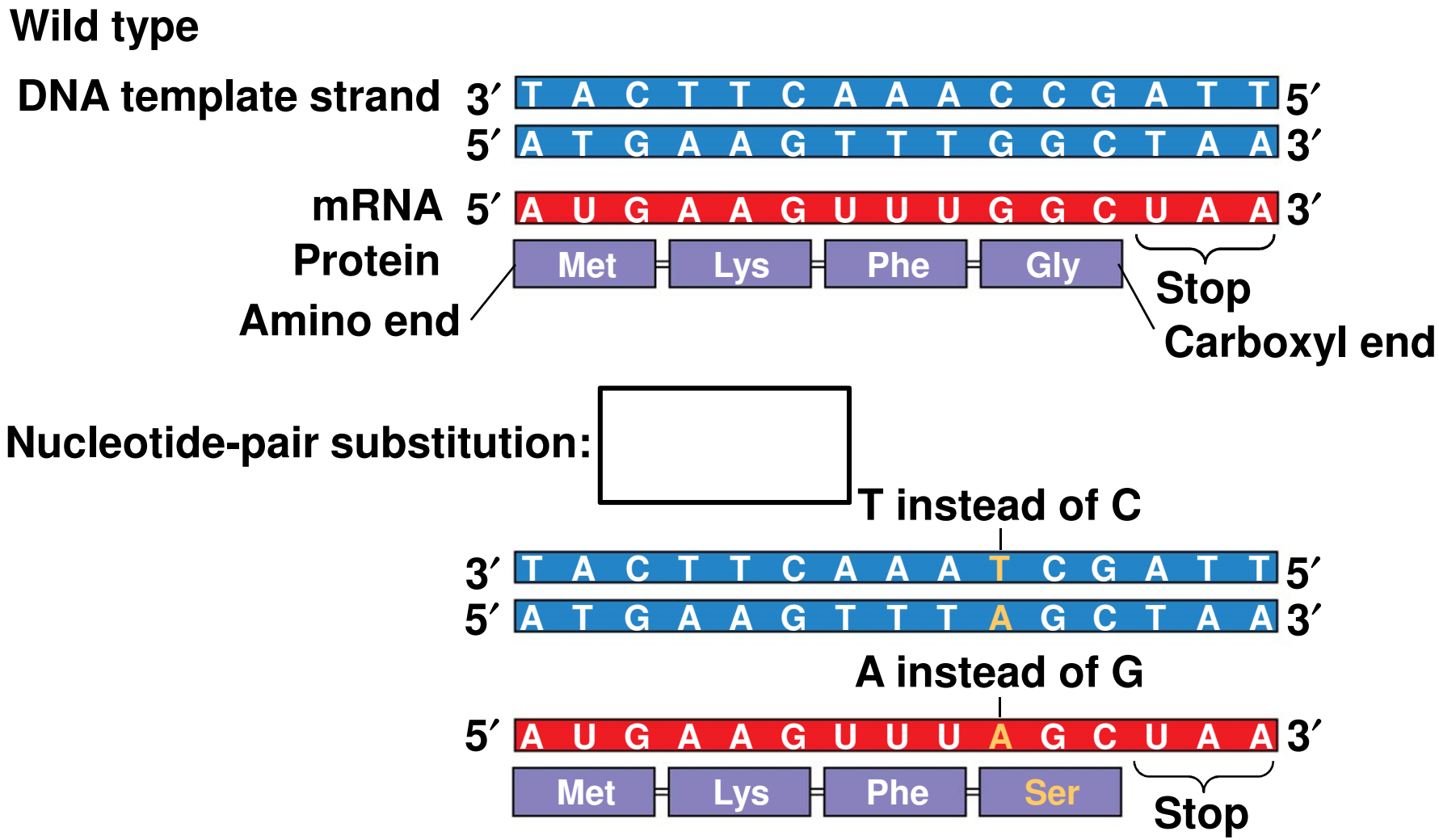
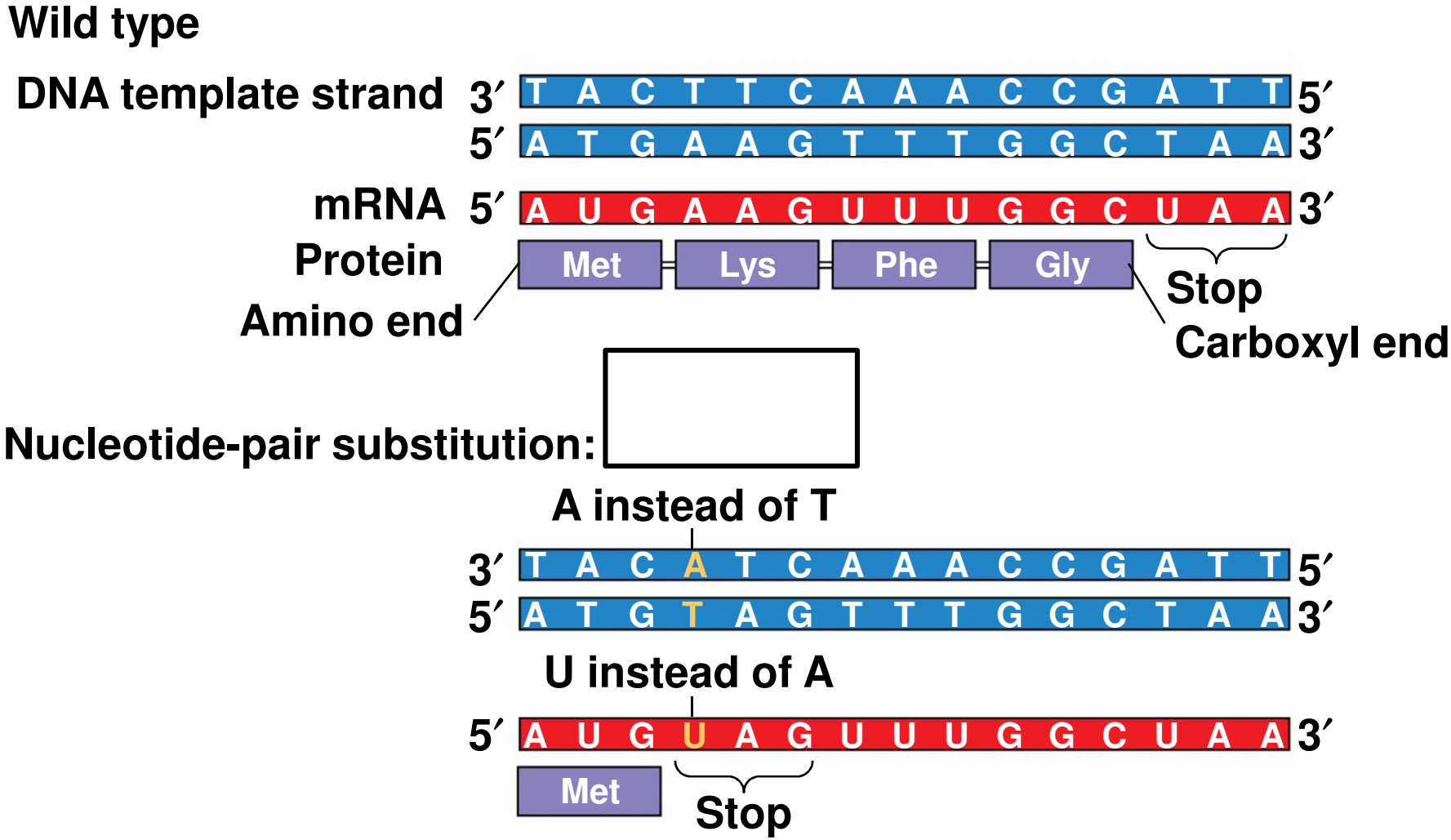


Figure 14.26c



Insertions and Deletions

- **Insertions** and **deletions** are additions or losses of nucleotide pairs in a gene
  - These mutations have a disastrous effect on the resulting protein more often than substitutions do
- Insertion or deletion of nucleotides may alter the reading frame of the genetic message, producing a **frameshift mutation**
  - All of the nucleotides downstream of the deletion or insertion will be improperly grouped into codons
  - Results will be extensive missense
  - Resulting protein is typically nonfunctional
- Note: Inserting or deleting nucleotides in multiples of 3 will NOT shift reading frame
  - But will insert or delete a single amino acid

Figure 14.26d

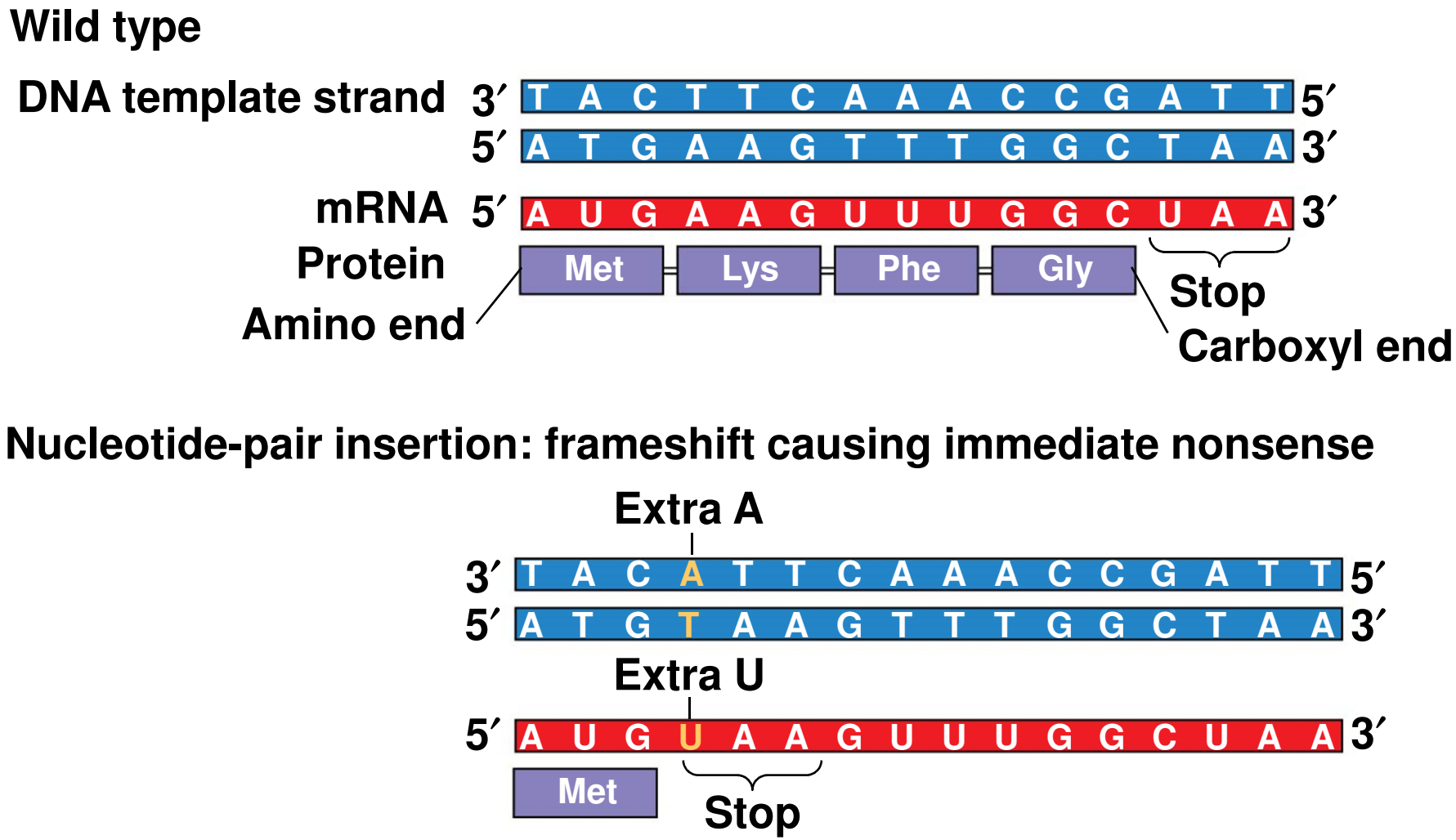


Figure 14.26e

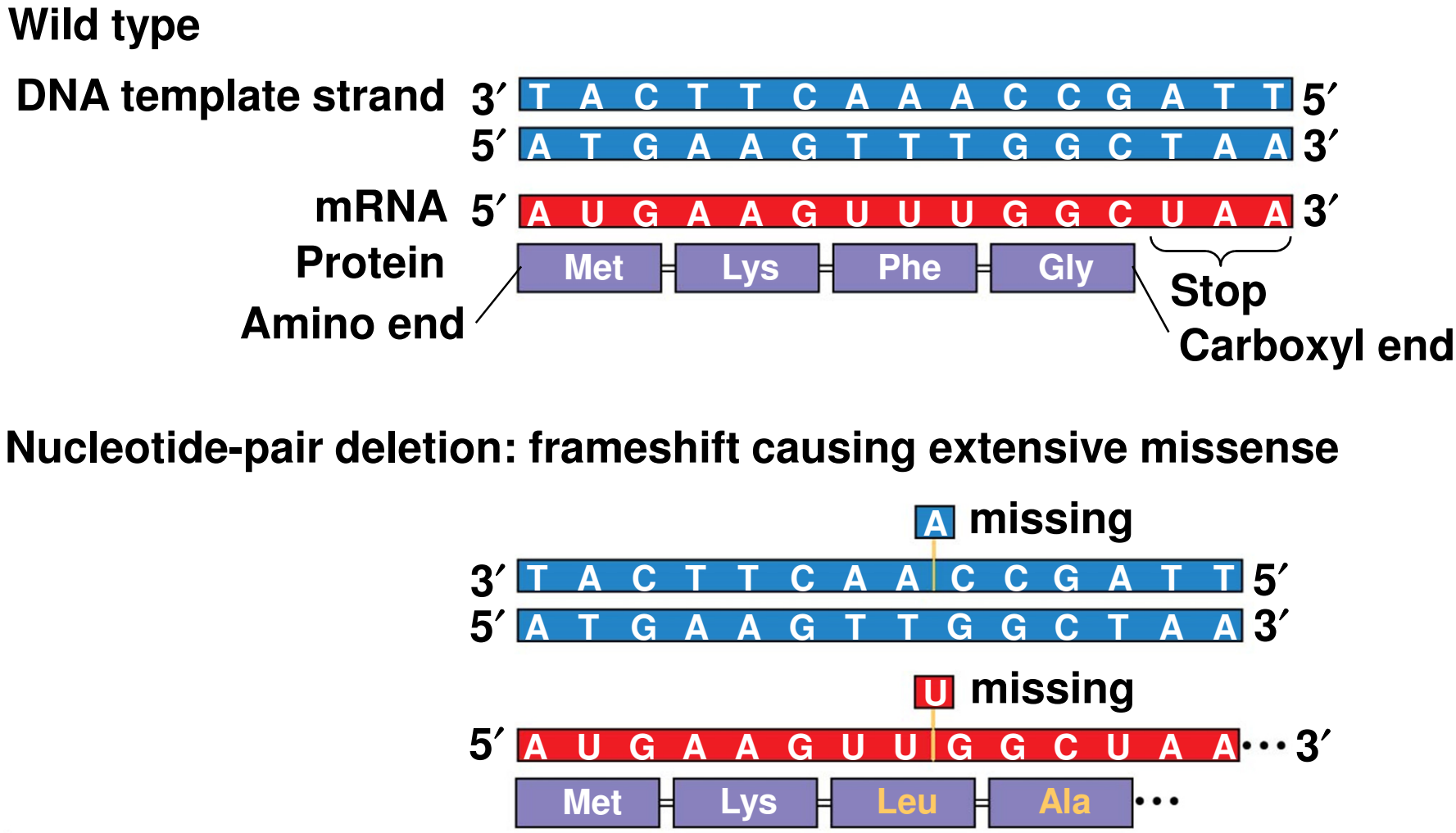
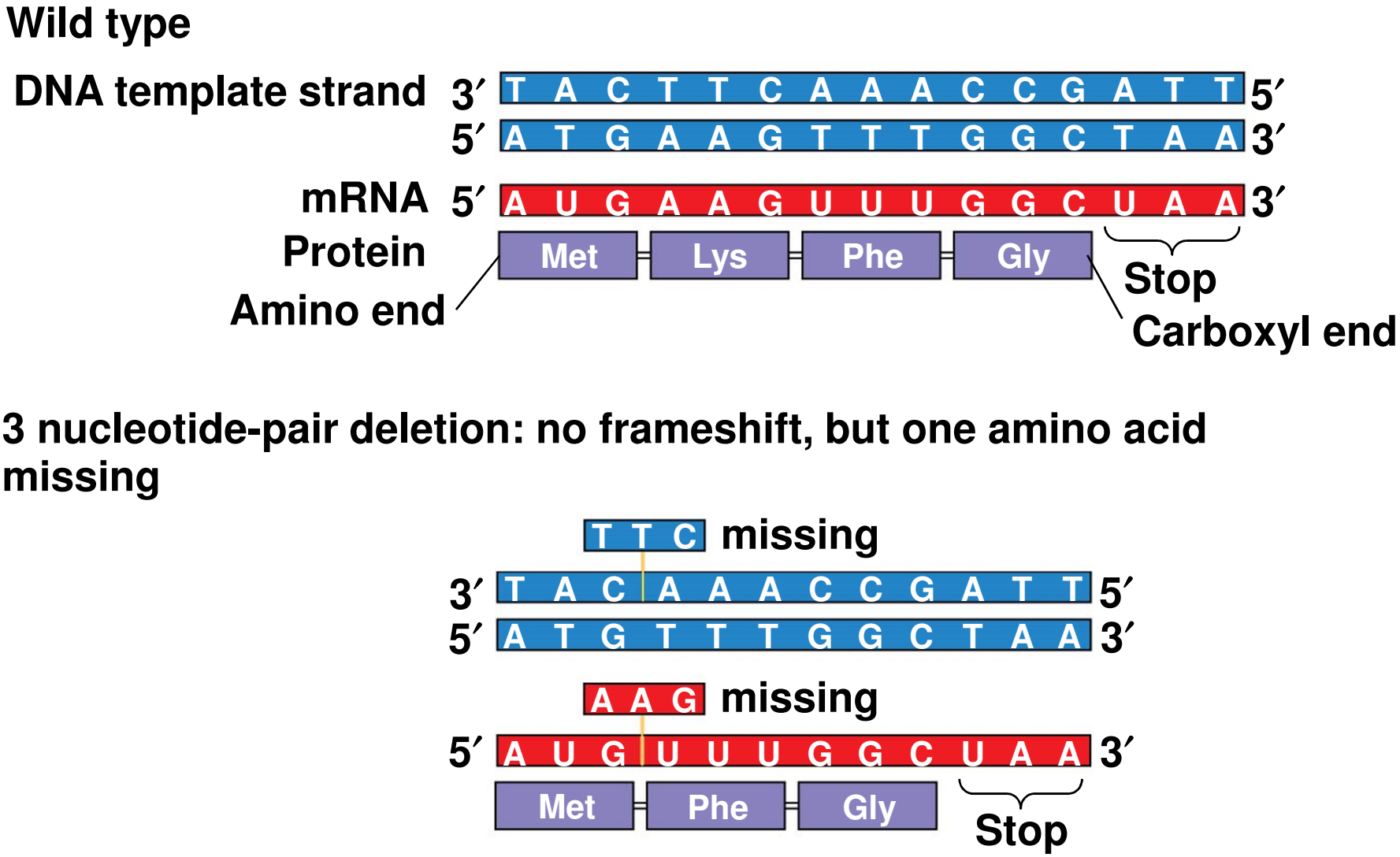


Figure 14.26f



Unit 6
Molecular Biology

Chapter 15: Regulation of Gene Expression

Differential Expression of Genes

- All of the cells in an organism have the same genome
  - They are just expressed differently!
- Prokaryotes and eukaryotes alter gene expression in response to their changing environment
- Gene expression is often regulated at the transcription stage

Gene Expression in Bacteria

- Natural selection has favored bacteria that produce only the gene products needed by the cell
- A cell can regulate production of enzymes on 2 levels
  1. *Feedback inhibition* (rapid response)
     - Enzyme inhibited by accumulation of its end product
     - Allows a cell to adapt to short-term fluctuations in the supply of a needed substance
  2. Gene regulation (longer-term response)
     - Transcription of the mRNA coding for the enzyme is switched on or off
- Gene expression in bacteria is controlled by a mechanism described as the *operon model*

Operons

- A group of functionally related genes can be coordinately controlled by a single "on-off switch"
- The regulatory "switch" is a segment of DNA called an **operator** usually positioned within the promoter
  - Controls access of RNA polymerase to the genes
- An **operon** is the entire stretch of DNA that includes the operator, the promoter, and the genes that they control
- A **repressor** can switch an operon off
  - Prevents gene transcription by binding to operator
  - No repressor = continuous transcription

- A **repressible operon** is one that is usually on
  - Binding of a **repressor** to the operator shuts off transcription
  - Generally function in anabolic pathways
    - Synthesize essential end products from raw materials
- An **inducible operon** is one that is usually off
  - A molecule called an **inducer** inactivates the repressor and turns on transcription
  - Generally function in catabolic pathways
    - Break down nutrients into simpler molecules

Gene Expression in Eukaryotes

- In multicellular organisms, regulation of gene expression is essential for cell specialization
  - Almost all the cells in an organism are genetically identical
  - Differences between cell types result from **differential gene expression**
    - The expression of different genes by cells with the same genome
- A common control point for gene expression is at transcription

Chromatin Packing

- **Chromatin** is a complex of DNA and protein in the nucleus of eukaryotes
- As a cell prepares for mitosis, its chromatin coils and folds up (condenses)
  - **Histone**
    - Protein around which DNA coils
  - **Nucleosome**
    - Bead-like unit of DNA packing
    - Consists of a segment of DNA wound twice around histones

Figure 13.21a

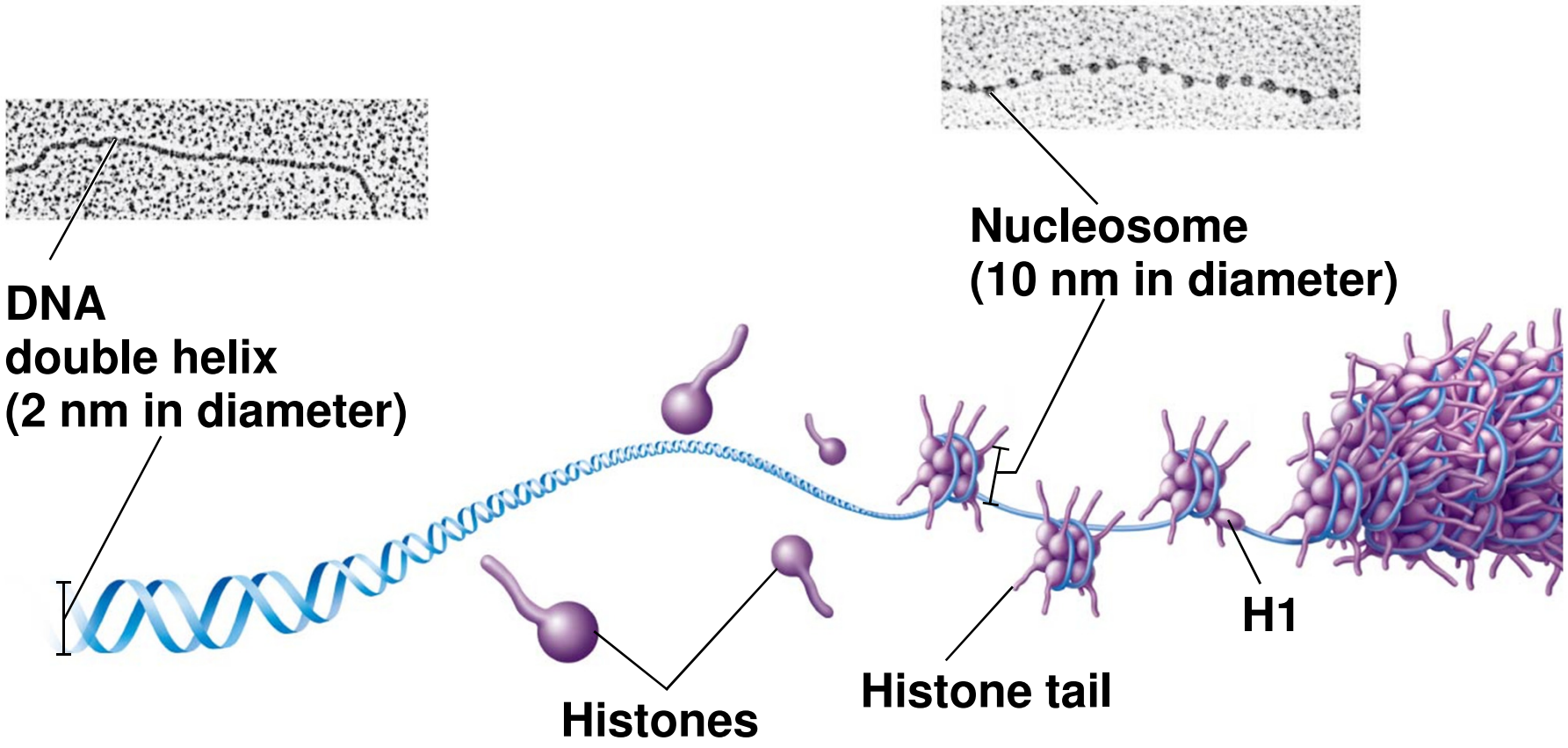
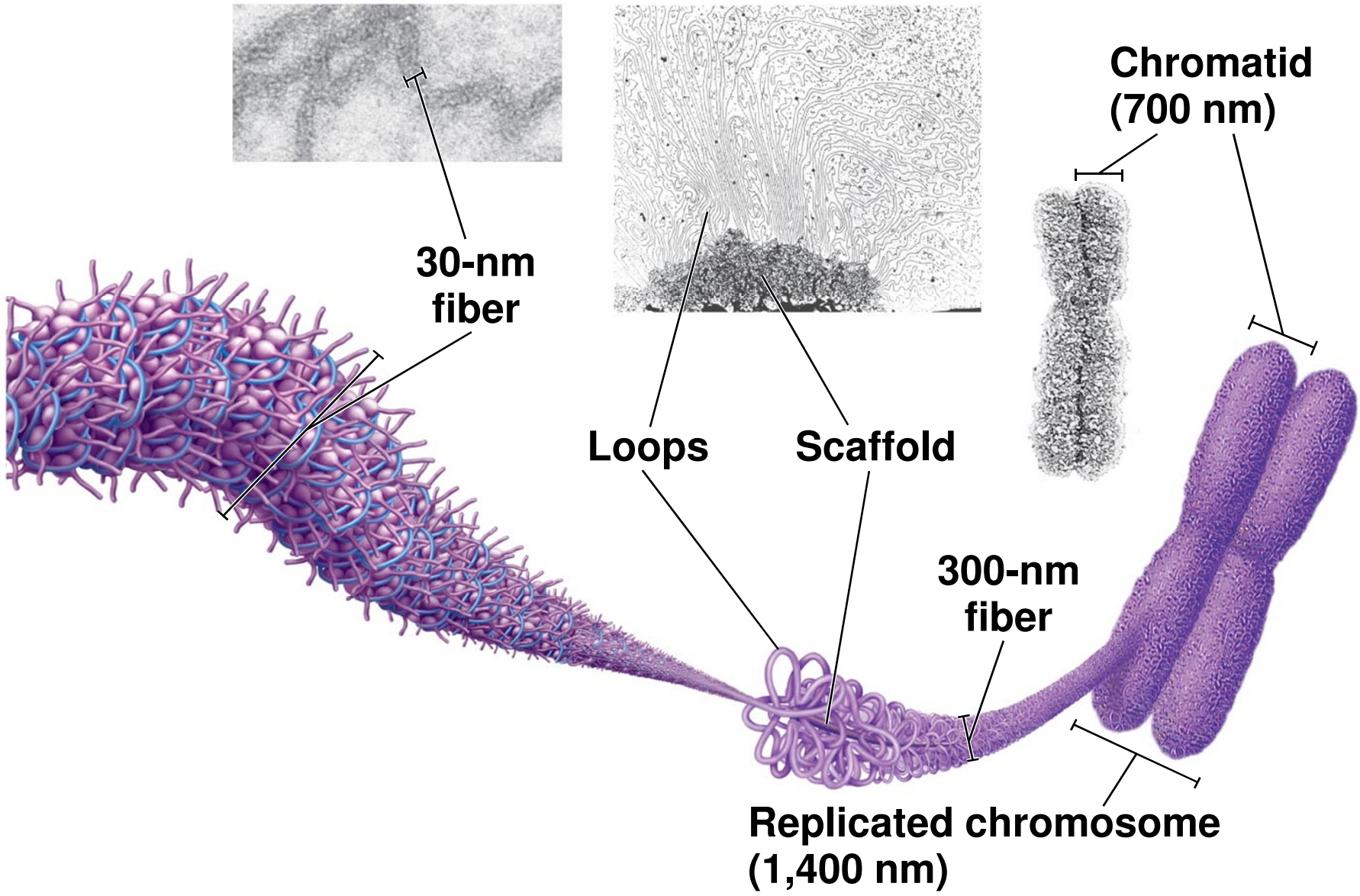


Figure 13.21b



- A condensed region may be loosened or modified as needed for various cell processes
- Condensed chromatin is called **heterochromatin**
  - Genes within highly condensed heterochromatin are usually not expressed
- Less compacted chromatin is called **euchromatin**
  - Looser packed euchromatin makes its DNA easier to transcribe
- **Acetylation** loosens chromatin structure
  - Thus promoting the initiation of transcription
- **Methylation** can condense chromatin
  - Leads to reduced transcription

Transcription Factors

- To initiate transcription, eukaryotic RNA polymerase requires the assistance of proteins called **transcription factors**
- Some transcription factors function as
  - **Activators** = bind to an enhancer and stimulate transcription of a gene
  - **Repressors** = inhibit expression of a particular gene

- Genes will be expressed if EITHER
  - Activator is present
  - Repressor is absent
- Genes will not be expressed if EITHER
  - Activator is absent
  - Repressor is present